

## Convenient surface functionalization of whole-Teflon chips with polydopamine coating

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This paper presents a convenient strategy to modify the surface of whole-Teflon microfluidic chips by coating the channel walls with a thin layer of polydopamine (PDA) film, which is formed by oxidation-induced self-polymerization of dopamine in alkaline solution. Two coating strategies, static incubation and dynamic flow, are demonstrated and used for tuning the physical and chemical properties of the coated channel walls. The functionalized surfaces were investigated with the contact angle, X-ray photoelectron spectroscopy, and atomic force microscopy measurements. The coating time was optimized according to the fluorescent intensity of the green fluorescent protein immobilized on the modified surface. Applications of the PDA-modified Teflon microchips in bioanalysis were demonstrated with a typical sandwich immunoassay. Moreover, long-term cell culture experiments on modified and native Teflon chips revealed that the chip biocompatibility can be greatly improved with PDA coating. The results indicate that the surface properties of the Teflon can be easily controlled by the PDA modification, thus greatly expanding the application scope of whole-Teflon chips for various chemical and biological research fields. © 2015 AIP Publishing LLC.

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### I. INTRODUCTION

Surface engineering of microfluidic devices could offer various functionalities for on-chip exploration, which is crucial for the success of applications in chemical analyses, bioassays, and cell-based experiments.<sup>1–3</sup> Polymeric materials, such as polydimethylsiloxane (PDMS), polymethyl methacrylate (PMMA), and polystyrene (PS), with their ease of fabrication and versatile post modification properties, are popular choices for researchers to manufacture microfluidic devices.<sup>4–7</sup> Recently, we developed a novel fabrication method to construct whole-Teflon microfluidic chips in fluorinated ethylene propylene (FEP) and perfluoroalkoxy (PFA) with integrated valves and pumps.<sup>8</sup> Compared with the widely used PDMS devices, Teflon chips offer unique advantages of having no small molecule adsorption and little adsorption of biomolecules, excellent anticorrosion, and compatibility with organic solvents; these merits make the whole-Teflon chip a potentially ideal platform for applications such as on-chip organic reactions and bioanalyses.<sup>9,10</sup> Native Teflon materials, however, are well known for their chemical inertness. It is very difficult to introduce functional groups to the surface, which greatly hinders wide applications of whole-Teflon chips.

Previously, two approaches have been exploited for modification of Teflon materials. The first one uses wet chemical etching, where sodium in liquid ammonia,<sup>11</sup> sodium naphthalene,<sup>12</sup> and FluoroEtch<sup>13</sup> activate Teflon surface and subsequently introduce functional groups. By treating with sodium naphthalene, the adhesion of epoxy resin<sup>12</sup> and copper<sup>14</sup> to Teflon surface

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was greatly improved. Besides, rich carboxyl groups were generated on FEP sheet by means of FluoroEtch, which facilitated the bonding between FEP sheet and APTES modified glass substrates via well-established carbodiimide crosslinking chemistry.<sup>13</sup> The second method employs high-energy sources including plasma, irradiation, and corona to activate the Teflon surface.<sup>15,16</sup> For example, Chu *et al.* adopted plasma treatment to change both the morphological and chemical structures of PTFE surface on which osteoblast<sup>17</sup> and mesenchymal stem cell<sup>18</sup> behaviors were well tailored. In addition, Lee *et al.* conducted sensitive DNA detections on functionalized FEP substrate by treating the inert surface with ion implantation and graft polymerization.<sup>19</sup> However, both methods have several limitations, making them unsuitable for *in situ* on-chip modifications of Teflon microchannels. For chemical treatment, it is hard to finely control the etching process because the etchants are highly reactive and dangerous.<sup>20</sup> Extreme care should be taken. For processes involving high energy, complicated, and expensive apparatus are required, which is unavailable for common chemistry and biological laboratories; in addition, these treatments often result in complex chemical composition and thus lead to difficulty in subsequent specified chemical modifications.<sup>21</sup> Both methods are only amenable to flat substrates and are very difficult to be used to modify the enclosed microchannels of microfluidic chips (because the bonding of whole-Teflon chips requires high temperature, which nullifies surface modifications before the bonding of the microchannels). Hence, a safe and convenient method to modify the channel surface of Teflon chips with good controllability and reproducibility is of great significance.

Inspired by the unique adhesion mechanism of mussel, Messersmith *et al.* proposed a universal route to coat polydopamine (PDA) film onto almost any kind of surface.<sup>22–25</sup> Due to its feasibility and versatility, this PDA coating process is proved to be a powerful tool for chemical and biomedical applications.<sup>26–29</sup> For instance, Lee *et al.* deposited PDA film on hydrophobic surfaces to construct surface tension confined droplet microfluidic system.<sup>30</sup> We coated PDA on to parafilm and hydrophobic glass to study the osteogenic stem cell differentiation and human adipose mesenchymal stem cell spreading and proliferation.<sup>31,32</sup> Jiang *et al.* exploited microcontact printing technique to realize the spatially defined cell and bacterial patterns by use of interaction between PDA and polyethylene glycol.<sup>33</sup> Here, we adapt the PDA film coating to modify the whole-Teflon microchannel walls and show some applications of the modified chips that are difficult with native Teflon chips. Compared with PDMS fabricated devices, these functionalized whole-Teflon chips can still maintain the advantage of organic solvent resistance (PDA coatings are stable in several commonly used organic solvents<sup>34</sup>). Furthermore, we can selectively modify certain parts of Teflon chips for particular applications (for example, the modified Teflon area can be used for molecular recognition or metal deposition, while the native surface for loss-free transportation of target analyte).

Two strategies of PDA coating, dynamic flow and static incubation, were investigated to generate different coating patterns inside the channels. Water contact angle analysis showed the hydrophilic property of the modified surface, while the surface chemical composition and morphology change were studied by X-ray photoelectron spectroscopy (XPS) and atomic force microscopy (AFM). The time for molecule immobilization on the surface was optimized to be 3 h with continuous flow, and a sandwich immunoassay was demonstrated on the modified Teflon chip. We also found that the biocompatibility of chip could be greatly improved by culturing HeLa cells and 3T3 cells, respectively, on native and PDA-modified microfluidic chips. These results show that whole-Teflon chips can be conveniently and safely modified with PDA coating, and this modification can greatly expand the applications of whole-Teflon chips in the chemical and biological research fields.

## II. MATERIALS AND METHODS

### A. Materials and equipment

We purchased PDMS prepolymer from GE Silicones, RTV615. Teflon FEP plates were obtained from Yuyisong, Inc. (Shanghai, China). Negative photoresist SU-8 2050 and developer were from MicroChem, Newton, MA. Green fluorescence protein (GFP), Eagle minimum

essential medium (MEM), Dulbecco's modified Eagle medium (DMEM), fetal bovine serum (FBS), 0.25% trypsin, and antibiotics penicillin/streptomycin were all from Gibco, Invitrogen, NY. LIVE/DEAD<sup>®</sup> Viability/Cytotoxicity Kit (L-3224) was purchased from Life Technologies, Thermo Fisher Scientific. All the other chemicals were from Sigma-Aldrich and were analytical grade and used without further purification.

Water contact angles were measured with a JC2000A contact angle analyzer with ultrapure deionized water (5  $\mu$ l). X-ray photoelectron spectroscopy was conducted with a Model PHI 5600. Atomic force microscopy characterization was performed on NanoScope Dimension 3100 under tapping mode. Bright field images were taken using Nikon microscope (AZ100), the fluorescence pictures were taken by the Nikon inverted fluorescence microscope (Eclipse Ti) equipped with a CCD camera (DS-Fi) using a green filter, and the fluorescence intensity were analyzed using NIS-BR software.

## B. Teflon chip fabrication

The fabrication of Teflon chips was followed with the work done by our previous work.<sup>8</sup> We first fabricated the SU-8 micropattern on silicon wafer, then 500  $\mu$ m thick PDMS (5:1 weight ratio of components A and B) layer was spin coated onto the pattern, and the PDMS master was fabricated after transferring this fully cured PDMS membrane to glass slide and further cured in a 250 °C oven (KSW5-12-A, Zhonghuan Experiment Electric Stove, China) for 1 h. The Teflon FEP plates were thermal molded at 265 °C for 5 min using a hot compressor (TM-101F, Taiming, Inc, China). After drilling holes for connection and washing with acetone by sonication, two FEP plates were thermal bonded in the oven (260 °C, 1 h).

## C. Polydopamine coating

We employed two methods to form polydopamine layer inside channel. One is to directly load the pre-mixed dopamine solution (2 mg/ml dopamine in 10 mM bicine buffer, pH = 8.5) into the microchannel, and incubated for certain time (which varies depending on coating method) at room temperature. The other approach was using continuous flow solution to coat the channel, the dopamine solution was mixed from bicine buffer (20 mM, pH = 8.5) and dopamine solution (4 mg/ml) using a Y-shaped chip under the same flow rate, and then it was immediately transported into Teflon chip. The coating process was maintained at a constant flow rate (5  $\mu$ l/min) at room temperature. When measuring the corresponding PDA thickness in either static or constant-flowing solutions, we functionalized the Teflon channel with the time ranging from 1 h to 5 h; when selectively modifying the Teflon channel surface to generate plug patterns and laminar flow patterns, we carried out the coating processes for 24 h and 12 h, respectively, to obtain thick PDA layer for facilitating optical observation. After PDA coating, we rinsed the channel surface thoroughly by infusing water at a constant flow rate (10  $\mu$ l/min) for 5 min using a syringe pump.

## D. Methods for contact angle measurement and PDA thickness measurement

In order to measure contact angle, we placed a PDMS cover slab (containing a channel with dimensions of 5 mm width and 70  $\mu$ m height) onto a Teflon flat substrate to form an enclosed channel. Then, this chip was sandwiched between two polymethylmethacrylate (PMMA) plates (fastened by screws) to assure that there was no leakage. To form PDA coatings, the dopamine solutions were either directly introduced into the channel (for static incubation) or constantly infused by a syringe pump at a constant flow rate of 125  $\mu$ l/min (for dynamic flow). After PDA coating, the channel was disassembled and the Teflon substrate was washed thoroughly using a wash bottle and dried by nitrogen blow. To test the stability of PDA coatings, we stored the coated Teflon substrate in a refrigerator (4 °C) in dark for four months.

In order to characterize PDA coating morphology, we adopted the same chip configuration that was used for contact angle measurement. Here, the PDMS cover slab contained the channels with dimensions of 40  $\mu$ m width and 70  $\mu$ m height. For dynamic flow, the solution

was maintained at a constant flow rate of 1  $\mu\text{l}/\text{min}$ . The coating time ranged from 1 h to 5 h. Before AFM examination, the Teflon substrate was washed thoroughly using a wash bottle and dried by nitrogen blow. Relatively smooth areas were selected for measuring PDA film thickness by AFM, thus excluding the interference of PDA nanoparticle.

### E. GFP immobilization and sandwich immunoassay

Teflon chips adopted in this work are composed of five parallel channels with the dimension of 200  $\mu\text{m}$  width and 70  $\mu\text{m}$  height. After the channels were coated by polydopamine and rinsed, we introduced GFP solution (3  $\mu\text{l}$ , 100  $\mu\text{g}/\text{ml}$ ) and incubated the chip for molecule immobilization for 3 h at room temperature. Before measuring the fluorescent intensity, the channels were rinsed with phosphate buffered saline (PBS) with 0.05% Tween-20 at a constant flow rate (5  $\mu\text{l}/\text{min}$ ) for 5 min. When performing sandwich immunoassay detection, the Teflon chips were coated by dopamine solution for 3 h at a constant flow rate (5  $\mu\text{l}/\text{min}$ ) and rinsed. The chips were loaded with anti-rabbit IgG (3  $\mu\text{l}$ , 200  $\mu\text{g}/\text{ml}$  in bicine buffer) at 4 °C overnight. After flushing the channels with PBS of 0.05% Tween-20 (5  $\mu\text{l}/\text{min}$ , 5 min) and PBS (5  $\mu\text{l}/\text{min}$ , 5 min), different concentrations of rabbit IgG (3  $\mu\text{l}$ , PBS) were loaded. The chips were kept at room temperature under humid environment for 30 min, and then the channels were washed with PBS with 0.05% Tween-20 (5  $\mu\text{l}/\text{min}$ , 5 min) and PBS (5  $\mu\text{l}/\text{min}$ , 5 min), and loaded with FITC labeled anti rabbit IgG (3  $\mu\text{l}$ , 200  $\mu\text{g}/\text{ml}$  in PBS), and fluorescent images were taken and analyzed after 30 min incubation and channel rinsing step (PBS with 0.05% Tween-20, 5  $\mu\text{l}/\text{min}$ , 5 min).

### F. Cell culture

Cervical cancer (HeLa) cell line and mouse embryonic fibroblast (NIH 3T3) cell line were maintained in MEM and DMEM, respectively. Both media were supplemented with 10% FBS, 100 U/ml penicillin, and 100  $\mu\text{g}/\text{ml}$  streptomycin. The cells were manipulated under sterile tissue culture hood and cultured in a humid incubator at 37 °C with 5%  $\text{CO}_2$ . After modification with polydopamine, the Teflon chips were washed and sterilized with 75% ethanol for 30 min. The cell passage was achieved with standard 0.25% trypsin solution and neutralized with cell culture medium. The cell suspension solution was centrifuged at 1250 rpm for 3 min and the cells were resuspended in the cell culture medium. After counting the cell number, the suspension was diluted to a desired density ( $2 \times 10^6$  cells/ml) and was loaded into the channel for further culture and observation. The phase contrast images of cell were taken from the channels with dimensions of 300  $\mu\text{m}$  width and 100  $\mu\text{m}$  height. We stained the cell by changing culture medium with PBS (containing 2  $\mu\text{M}$  calcein AM and 4  $\mu\text{M}$  EthD-1) inside wide channels (1 mm width and 500  $\mu\text{m}$  height). The quantitative experiments of cell culture were performed three times, and the cell area and cell number were measured using Image J.

## III. RESULTS AND DISCUSSION

### A. Modification of Teflon chip

According to the previous reports,<sup>23,35</sup> both pH and oxidant in solution play critical roles during the self-polymerization reaction of dopamine. The typical PDA coating is achieved by immersing the substrates in bulk solution of dopamine under continuously stirring in an open environment, and the coating process would gradually cease due to the depletion of dopamine after about 12 h.<sup>23</sup> Thus, to deposit thicker layer of PDA film, multiple coating steps are required. But when coating channels in microfluidic channels, there are some differences due to two reasons: the solution in the channel cannot be stirred thoroughly and the consumed oxygen cannot be replenished promptly, both leading to a low efficiency of PDA coating. Herein, to overcome these problems, two methods are applied to modify channel surfaces in our study: static incubation (Figure 1(a)) and dynamic flow (Figure 1(b)). In the static incubation method, the channels were simply loaded with pre-mixed dopamine solution and kept for certain time to generate PDA surface coating. In this scheme, potassium chlorate was doped in the solution as

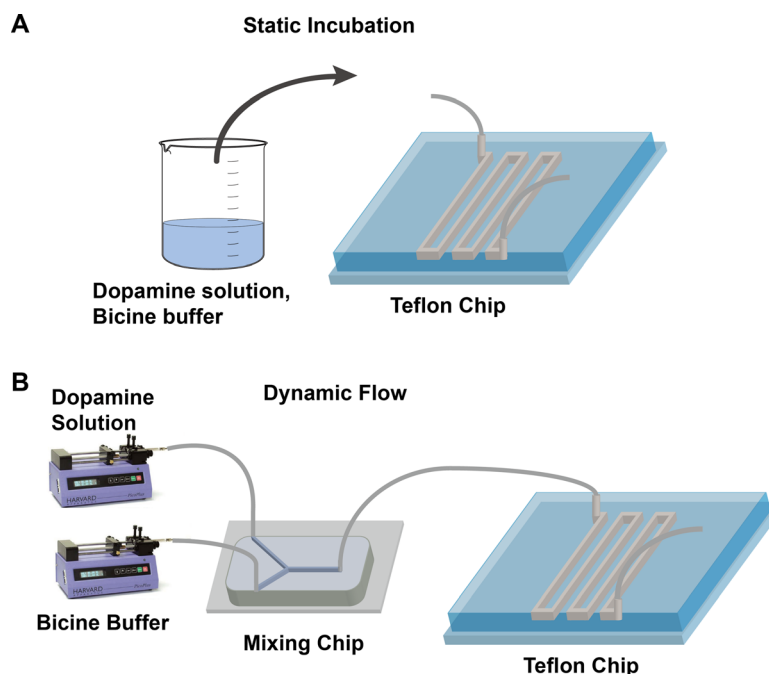


FIG. 1. Methods for modifying Teflon chips: (a) Static incubation: the pre-mixed dopamine solution (2 mg/ml in 10 mM bicine buffer, pH = 8.5) was directly introduced into the Teflon channel and incubated for defined time; (b) dynamic flow: the mixed dopamine solution was prepared from dopamine solution (4 mg/ml) and bicine buffer (20 mM, pH = 8.5) using a Y-shaped mixing chip and then this mixed solution was simultaneously delivered to Teflon chips.

oxidant<sup>35</sup> to compensate for the deficiency of oxygen in the channel. Thicker PDA layer can thus be formed by repeating this static incubation step. This method can also generate patterns in the channel (Figure 2(a)) by introducing gas plugs as spacer to separate the solution. In the dynamic flow method, it involves two steps: the first step is to obtain mixed dopamine solution by a Y-shaped microfluidic chip with one inlet filled with a dopamine solution and the other inlet with a bicine buffer; and the second step is to introduce this prepared solution into Teflon channel by connecting mixing chip and Teflon chip to allow for dynamic surface coating, thus avoiding the tedious steps of changing solution. In addition, we formed laminar flow pattern (Figure 2(b)) inside the channel using the above protocol by separately introducing *in situ*

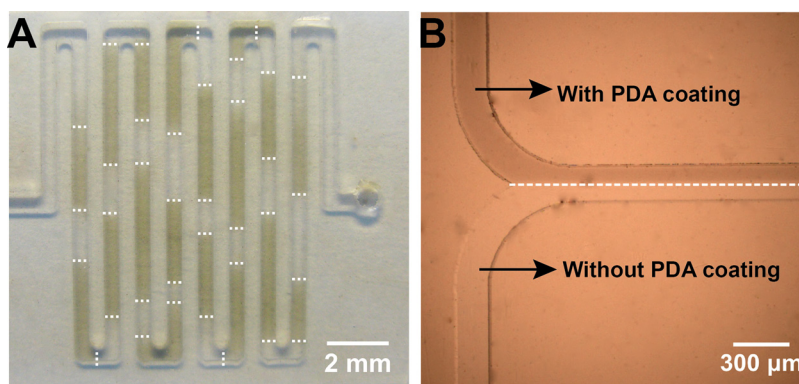


FIG. 2. Bright field images of PDA modified Teflon chip channels with different patterns (the dark areas are covered by PDA film and we use dashed lines to display the boundary). (a) Plug pattern: dopamine droplets (2 mg/ml dopamine, 0.65 mg/ml potassium chlorate, 10 mM bicine buffer, pH = 8.5) were introduced into the channel and spaced by air, then the chip was incubated for 24 h at room temperature. (b) Laminar flow pattern: the *in situ* mixed dopamine solution and water were introduced separately into the Teflon channel with a T-shaped pattern at the same flow rate at room temperature for 12 h. We coated the channel for a longer time in order to obtain obvious color change.



mixed dopamine solution and water into the inlet of T-shaped Teflon channel and maintaining the flow rate for 12 h.

## B. Surface characterization

After modification, the FEP surfaces with the PDA film coating show significant color change from translucent white to brown as shown in Figures 2(a) and 2(b). By increasing the coating time, the color becomes darker as the growth of PDA film. Comparing with the native FEP (with an average contact angle of  $105.6^\circ$ , Figure 3(a)), the PDA-modified surfaces are more hydrophilic (static incubation method:  $45.4^\circ$ , Figure 3(b); dynamic flow:  $42.2^\circ$ , Figure 3(c)). We further find that the water contact angle on surfaces treated with two methods by diverse time showed no distinct difference (within  $5^\circ$ ) due to the full coverage of the underlying substrates by the PDA film. The increase of hydrophilicity inside the channel facilitates the introduction of aqueous solutions without trapping air bubbles. This hydrophilic surface property is stable for at least several months under humid environments or in neutral pH solutions, which agrees with previous reports.<sup>36</sup>

In comparison, XPS and AFM measurements can offer details in the surface chemical composition and morphology, respectively. As shown in Figure 3(d), the wide XPS spectrum of native FEP indicates that the surface only contains fluorine and carbon. After PDA modification, chemical composition changes significantly. For surface modified by 3 h static incubation, the fluorine peak intensity decreases substantially, and all carbon, oxygen, and nitrogen peaks rise obviously. For surface modified with 3 h dynamic flow, the fluorine signal can be barely observed. In addition, Figure 3(e) quantitatively presents the change of surface element contents. With the static incubation for 3 h, there is still  $\sim 23.7\%$  mol of fluorine; while with 3 h dynamic flow modification, the fluorine peak can hardly be seen and the nitrogen/oxygen ratio is consistent with that of polydopamine. The composition differences with the two

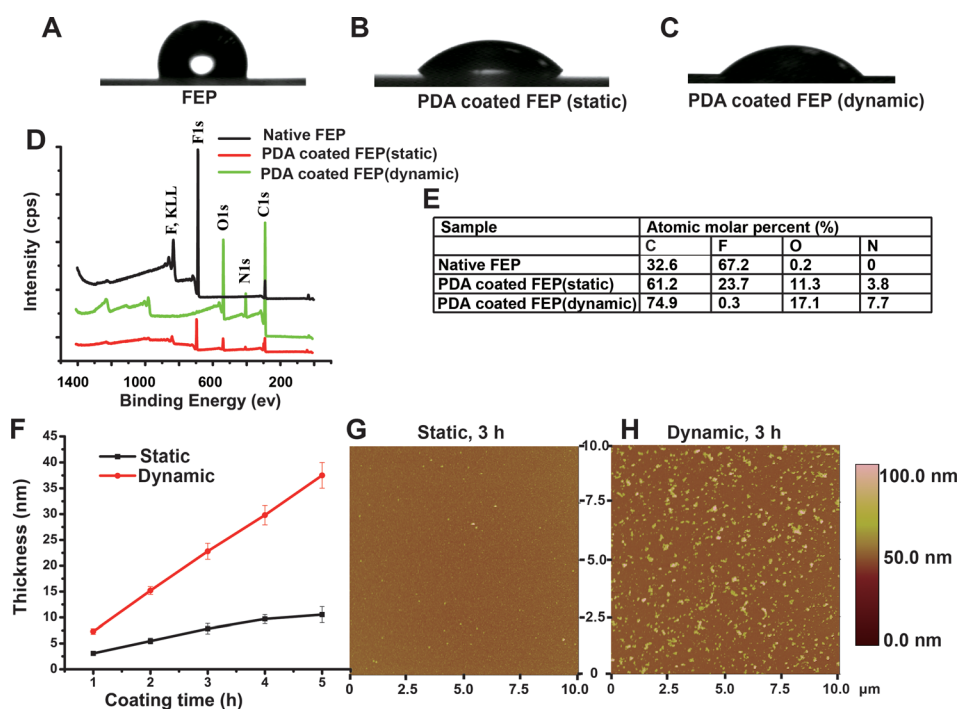


FIG. 3. Surface characterization of PDA coated FEP surface. Water contact angle analysis on (a) native FEP surface, on (b) PDA coated FEP surface by static incubation for 3 h, and on (c) PDA coated FEP surface by dynamic flow for 3 h. (d) XPS spectra of native and modified FEP surface. (e) Changes of surface chemical element composition. (f) Changes of PDA film thickness under different coating method with different time ( $n = 3$ ). (g) AFM characterization of modified FEP surface by static incubation for 3 h. (h) AFM characterization of modified FEP surface by dynamic flow for 3 h.

methods are caused by diverse PDA thicknesses because when the PDA film is less than 10 nm thick, underlying substrate can also give signals in XPS measurement.<sup>23</sup>

We also studied the thickness of the PDA film on the channel walls with different coating time using AFM. Compared with PDA coating in bulk solutions,<sup>23</sup> the film growth rate with static incubation is slower while the PDA film grows much faster with dynamic flow. This fast film growth is contributed from two reasons: large surface-to-volume ratio and continuous flow of dopamine solution in microchannels. As shown in Figures 3(g) and 3(h), surfaces that are treated with the two strategies show very different surface morphology: the static incubation gives smooth surface coating, while the dynamic flow method yields a rough coating with PDA nanoparticles scattering around. These PDA nanoparticles are built upon the interaction of dopamine solution (containing monomer molecule) and PDA film during the dynamic coating process, which is consistent with the previous report.<sup>37</sup> Apart from the coating time and method, initial dopamine concentration would also influence the surface morphology. It is expected that lower dopamine concentration would lead to thinner and smoother surfaces, while higher concentration would result in thicker and rougher PDA coatings.<sup>38</sup>

### C. Coating time optimization

The freshly prepared PDA film shows ready reactivity towards amine or thiol group-contained molecules such as trypsin,<sup>39</sup> amine-DNA,<sup>40</sup> and amine-PEG<sup>23</sup> by forming covalent bond via Michael addition or Schiff base formation under mild conditions.<sup>41</sup> Therefore, the Teflon channel surface can be activated for various molecule grafts in a very simple and convenient way. Here, we choose the dynamic flow modification method for the following reasons: higher coating efficiency as validated by the film thickness growth; more binding sites (which is confirmed by AFM analysis above and our previous publication<sup>32</sup> because more molecules can be immobilized on rough surfaces); and better reproducibility because of no depletion of oxygen in the surrounding solution.

We also optimized the coating time for optical detection because thick PDA film is not transparent in the UV and visible region.<sup>42</sup> GFP, a widely used fluorescent protein with abundant amine and thiol group outside its barrel structure<sup>43</sup> that can be used for molecule immobilization to PDA via Michael addition, is selected as model molecule for this optimization process. As shown in Figure 4(a), the distribution of fluorescence emission is even throughout the channel; the edges show higher fluorescent intensity due to the coating on the sidewall. Figure 4(b) shows the fluorescent intensity change versus PDA coating time (Figure 4(b)). Fluorescent intensity keeps increasing within the first three hours, followed by gradual decrease with longer coating time. This phenomenon is attributed from two aspects: a thicker PDA film

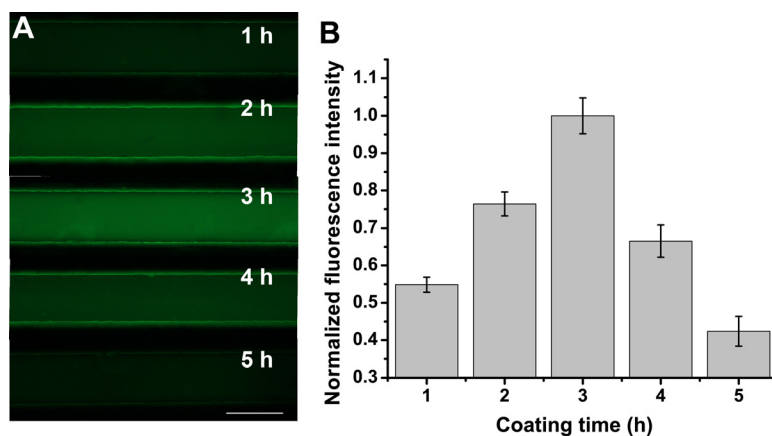


FIG. 4. Immobilization of GFP on FEP channel with PDA coated. (a) Fluorescent images of GFP immobilization on PDA modified FEP channel by different coating time. (b) Comparison of fluorescent intensity on channel coated with different time, the fluorescence is normalized to 1 according to 3 h of modification ( $n = 4$ ). Scale bar = 200  $\mu\text{m}$ .

provides more reaction sites for GFP immobilization, resulting increased fluorescence with PDA coating time; and, if the PDA film is too thick, both excitation and fluorescent lights are blocked by the PDA layer (both top and bottom part of the channels are coated with PDA), which leads to weaker signals. Therefore, we select 3 h as the optimized coating time for subsequent immunoassay.

#### D. Sandwich immunoassay

Microfluidic immunoassay shows optimistic prospect for clinical diagnostics due to the advantages of miniaturized sample consumption, potential high-throughput manner, and enhanced reaction efficiency.<sup>44</sup> To validate the feasibility of PDA-coated Teflon chips as bio-sensor platform for immunologic diagnosis, we performed a sandwich immunoassay of IgG detection on a PDA-modified FEP chip. The PDA layer serves as a reactive platform for immobilizing antibody through covalent bonding, which presents much better stability than electrostatic adsorption.<sup>45</sup> Introduction of the reactants and detection reagents was realized by simply dropping them at the channel inlet and then the whole channel would be filled automatically by the aqueous solution due to the capillary force induced by hydrophilic surface. During the immunoassay, the PDA-coated channels were loaded with anti-rabbit IgG for capturing antibody immobilization. After washing off the non-specifically bound antibody, target analyte (rabbit IgG), was introduced and incubated for antigen-antibody binding. Then detecting antibody (FITC-conjugated rabbit IgG) was loaded and specifically bounded to analyte. The fluorescent images of antibody-immobilized Teflon channels after incubating with antigen at different concentration are shown in Figures 5(a)–5(f). The detected fluorescence intensity gradually rises with the increase of concentration of rabbit IgG, while the fluorescence signal is almost negligible when no target analyte was introduced. By plotting the fluorescence intensity versus concentration curve, we obtained a calibration curve with a linear relation ( $R^2 = 0.992$ , Figure 5(g)) from 0 to 12.5  $\mu\text{g/ml}$ . Due to the active catechol groups in PDA layer, our method is quite simple and highly efficient because no cross-linking reagents is required. The simple modification and detection procedures suggested the PDA-modified Teflon chip can be utilized as a promising platform for diagnosis applications.

#### E. Cell culture

Microfluidic techniques have been constantly promoted to provide revolutionary platforms for the establishment of advanced cell culture models, among which the careful selection of appropriate substrate material is of vital importance.<sup>46</sup> Generally, non-wetting substrates, such as PE and PTFE, are not favorable for cell adhesion and proliferation.<sup>47</sup> In our previous work,<sup>8</sup> cells adhered to the surface of PFA channels that were pretreated with concentrated sodium hydroxide overnight, which is time consuming with involvement of corrosive reagents. Our above results proved that PDA coating can greatly improve the wetting properties of FEP surface, but the cell viability and activity on PDA-coated FEP channels are still needed to be investigated. Here, we simply deposited PDA film onto the channel to facilitate the cell adhesion and proliferation. After the cells were detached and diluted to the desired concentrations, the native and modified FEP channel were filled with cell suspension without trapping bubbles and cultured for 24 h in an incubator. The cell adhesion and cell proliferation on the PDA-coated surface of FEP channels were inspected under bright-field microscopy. As shown in Figures 6(a) and 6(c), neither 3T3 cell nor HeLa cell can adhere and proliferate well inside the native FEP channel. Most of these cells were in a globular shape and could be easily flushed out with flow. At channel corners, a few HeLa cells could spread a little probably because of the roughness of corner surface.<sup>48</sup> While in the PDA modified channels, the morphology of HeLa and 3T3 cells was much healthier: they spread extensively and attached well to the channel (Figures 6(b) and 6(d)). We further quantitatively evaluated the spread and adhesion of cell. Figure 7(a) shows the fluorescence images of cells stained by a LIVE/DEAD kit, only live cells were able to remain inside Teflon channel, while the dead cells were flushed out when introducing staining solution. The average surface area per cell (both HeLa and 3T3



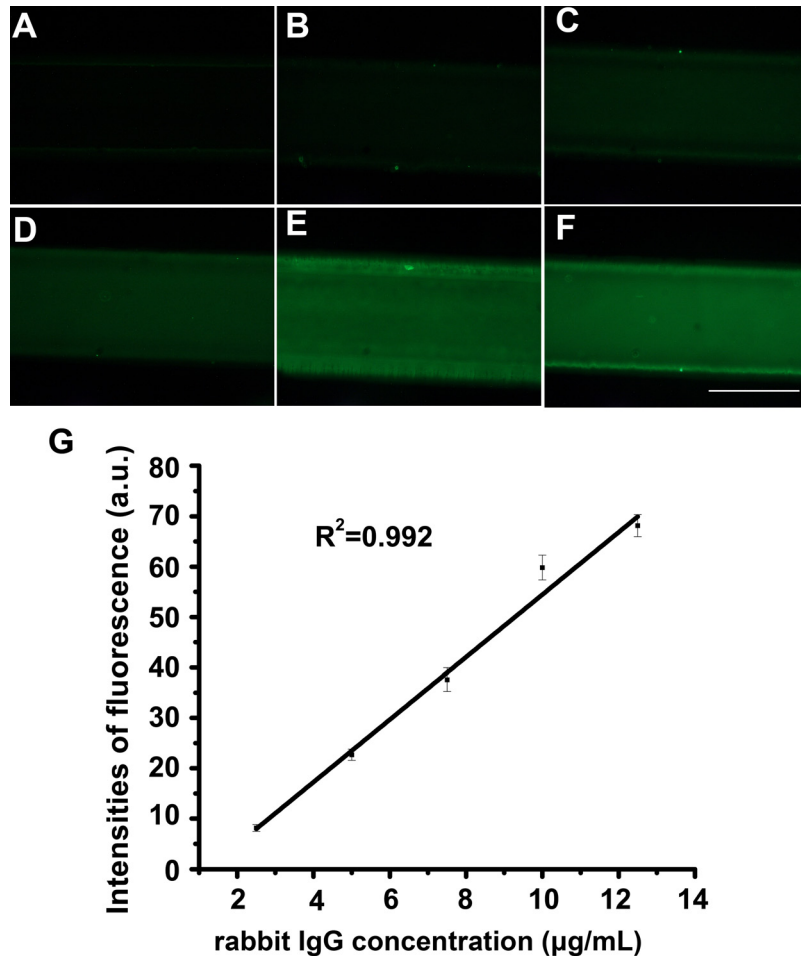


FIG. 5. Immunoassay detection of rabbit IgG. Fluorescence images of different concentrations of rabbit IgG: (a) 0  $\mu\text{g/ml}$ , (b) 2.5  $\mu\text{g/ml}$ , (c) 5  $\mu\text{g/ml}$ , (d) 7.5  $\mu\text{g/ml}$ , (e) 10  $\mu\text{g/ml}$ , (f) 12.5  $\mu\text{g/ml}$ . (g) Calibration plot of fluorescence intensity at different IgG concentrations with background subtraction ( $n = 4$ ). Scale bar = 200  $\mu\text{m}$ .

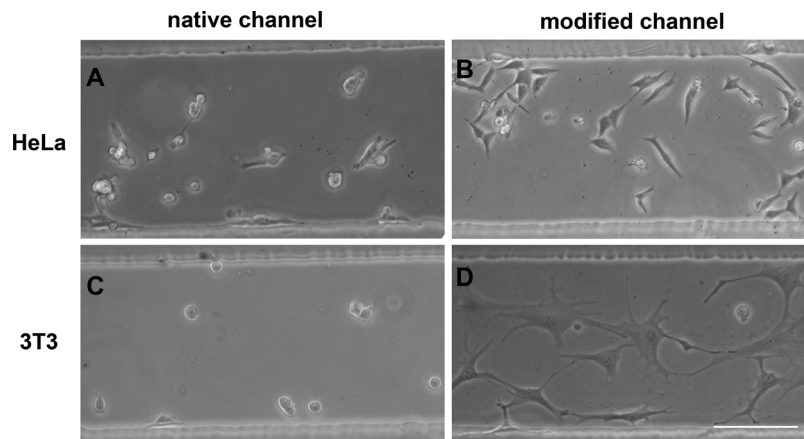


FIG. 6. Cell proliferation for 24 h inside modified and native Teflon channels. Phase contrast images of HeLa cell culture inside: (a) native channel, (b) PDA coated channel. Phase contrast images of 3T3 cell culture inside: (c) native channel, (d) PDA modified channel. Scale bar = 150  $\mu\text{m}$ .

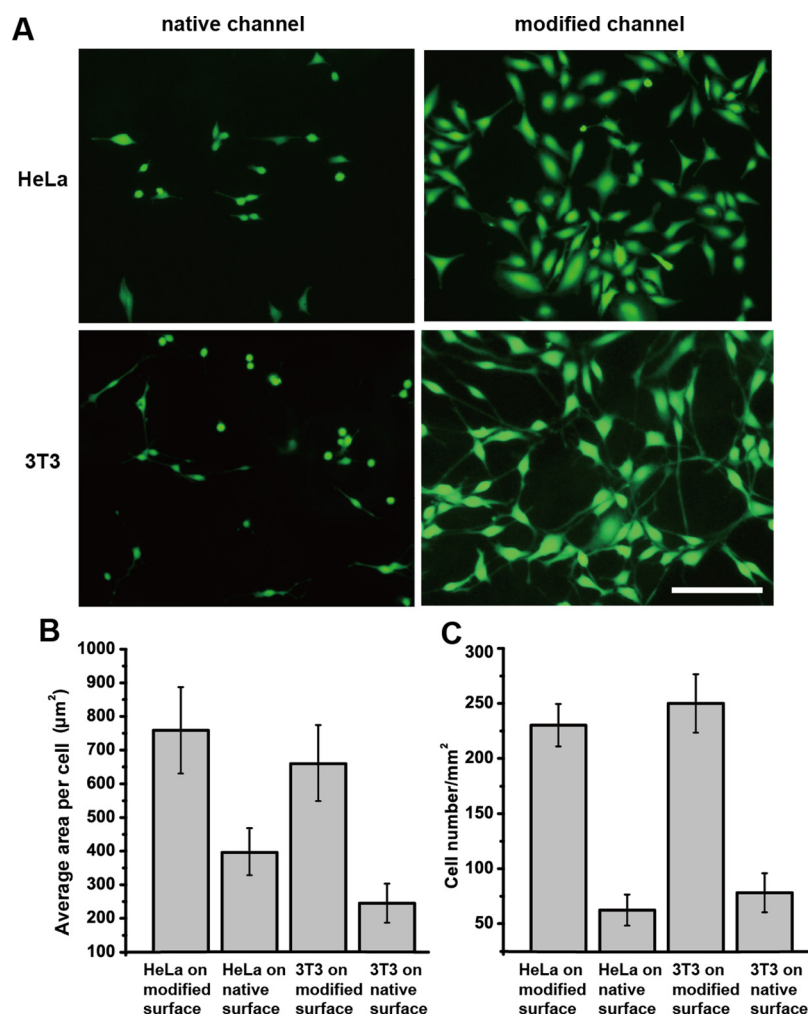


FIG. 7. Quantitative analysis of cell proliferation for 24 h on native and modified whole-Teflon chip. (a) Live/DEAD staining of HeLa and 3T3 cell. (b) Average area per cell. (c) Live cell density. Scale bar = 200  $\mu\text{m}$ .

cells) on modified surfaces was nearly twice as large as that of the cells on the native surface (Figure 7(b)). The cell densities inside PDA-coated channels were much higher (Figure 7(c)). All these results validate the improved biocompatibility of the modified FEP chip. The PDA-modified whole-Teflon chips with improved biocompatibility should be used for various cell cultures.

#### IV. CONCLUSIONS

We introduced a simple and convenient method to modify the channel surface of Teflon chips, which is accomplished by forming adherent polydopamine film onto the channel wall via the self-polymerization of dopamine. This PDA film shows great stability and reactivity towards various biomolecules that have terminal amine and thiol groups. After optimizing the coating time, this modified Teflon chip can be used as immunoassay platforms and shows improved biocompatibility. Compared with previously reported modification methods, this strategy is processed in a mild and convenient fashion without employing dangerous chemical etchants or expensive plasma instruments. Microfluidic chip surfaces that are fabricated with other materials, including PDMS, PMMA, and glass, can also be tuned by this dopamine coating schemes.

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